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We claim:

1. A flow electroporation device comprising:
a chamber for containing a suspension of cells to be electroporated;
5 the chamber being at least partially defined by opposing oppositely chargeable electrodes; and
wherein the thermal resistance of the chamber is less than approximately 10°C per Watt.
- 10 2. The flow electroporation device of Claim 1, wherein the thermal resistance of the chamber is less than approximately 9.5°C per Watt.
- 15 3. The flow electroporation device of Claim 1, wherein the thermal resistance of the chamber is less than approximately 9°C per Watt.
- 20 4. The flow electroporation device of Claim 1, wherein the thermal resistance of the chamber is less than approximately 8.5°C per Watt.
- 25 5. The flow electroporation device of Claim 1, wherein the thermal resistance of the chamber is less than approximately 9.5°C per Watt.
- 30 6. The flow electroporation device of Claim 1, wherein the thermal resistance of the chamber is less than approximately 8°C per Watt.
7. The flow electroporation device of Claim 1, wherein the thermal resistance of the chamber is less than approximately 7.5°C per Watt.

8. The flow electroporation device of Claim 1, wherein the thermal resistance of the chamber is less than approximately 7°C per Watt.

5 9. The flow electroporation device of Claim 1, wherein the thermal resistance of the chamber is less than approximately 6.5°C per Watt.

10 10. The flow electroporation device of Claim 1, wherein the thermal resistance of the chamber is less than approximately 6°C per Watt.

11. The flow electroporation device of Claim 1, wherein the thermal resistance of the chamber is less than approximately 5.5°C per Watt.

15 12. The flow electroporation device of Claim 1, wherein the thermal resistance of the chamber is less than approximately 5°C per Watt.

20 13. The flow electroporation device of Claim 1, wherein the thermal resistance of the chamber is less than approximately 4.5°C per Watt.

14. The flow electroporation device of Claim 1, wherein the thermal resistance of the chamber is less than approximately 4°C per Watt.

25 15. The flow electroporation device of Claim 1, wherein the thermal resistance of the chamber is less than approximately 3.5°C per Watt.

30 16. The flow electroporation device of Claim 1, wherein the thermal resistance of the chamber is less than approximately 3°C per Watt.

17. The flow electroporation device of Claim 1, wherein the thermal resistance of the chamber is less than approximately 2.5°C per Watt.

5 18. The flow electroporation device of Claim 1, wherein the thermal resistance of the chamber is less than approximately 2°C per Watt.

10 19. The flow electroporation device of Claim 1, wherein the thermal resistance of the chamber is less than approximately 1.5°C per Watt.

20. The flow electroporation device of Claim 1, wherein the thermal resistance of the chamber is approximately 0.1°C per Watt to 4°C per Watt.

15 21. The flow electroporation device of Claim 1, wherein the thermal resistance of the chamber is approximately 1.5°C per Watt to 2.5°C per Watt.

20 22. A method of electroporating a cell comprising flowing a suspension of cells to be electroporated through an electric field in a flow chamber, the electric field being produced by opposing oppositely charged electrodes at least partially defining the flow chamber, wherein the thermal resistance of the flow chamber is less than approximately 10°C per Watt.

25 23. The method of Claim 22, wherein the thermal resistance of the flow chamber is less than approximately 9.5°C per Watt.

30 24. The method of Claim 1, wherein the thermal resistance of the flow chamber is less than approximately 9°C per Watt.

25. The method of Claim 1, wherein the thermal resistance of the flow chamber is less than approximately 8.5°C per Watt.

5 26. The method of Claim 1, wherein the thermal resistance of the flow chamber is less than approximately 9.5°C per Watt.

27. The method of Claim 1, wherein the thermal resistance of the flow chamber is less than approximately 8°C per Watt.

10 28. The method of Claim 1, wherein the thermal resistance of the flow chamber is less than approximately 7.5°C per Watt.

29. The method of Claim 1, wherein the thermal resistance of the flow chamber is less than approximately 7°C per Watt.

15 30. The method of Claim 1, wherein the thermal resistance of the flow chamber is less than approximately 6.5°C per Watt.

20 31. The method of Claim 1, wherein the thermal resistance of the flow chamber is less than approximately 6°C per Watt.

32. The method of Claim 1, wherein the thermal resistance of the flow chamber is less than approximately 5.5°C per Watt.

25 33. The method of Claim 1, wherein the thermal resistance of the flow chamber is less than approximately 5°C per Watt.

34. The method of Claim 1, wherein the thermal resistance of the flow chamber is less than approximately 4.5°C per Watt.

30 35. The method of Claim 1, wherein the thermal resistance of the flow chamber is less than approximately 4°C per Watt.

36. The method of Claim 1, wherein the thermal resistance of the flow chamber is less than approximately 3.5°C per Watt.

5 37. The method of Claim 1, wherein the thermal resistance of the flow chamber is less than approximately 3°C per Watt.

38. The method of Claim 1, wherein the thermal resistance of the flow chamber is less than approximately 2.5°C per Watt.

10 39. The method of Claim 1, wherein the thermal resistance of the flow chamber is less than approximately 2°C per Watt.

40. The method of Claim 1, wherein the thermal resistance of the flow chamber is less than approximately 1.5°C per Watt.

15 41. The method of Claim 1, wherein the thermal resistance of the flow chamber is approximately 0.1°C per Watt to 4°C per Watt.

20 42. The method of Claim 1, wherein the thermal resistance of the flow chamber is approximately 1.5°C per Watt to 2.5°C per Watt.

43. A flow electroporation device comprising:
walls defining a flow channel having an electroporation zone configured to receive and to transiently contain a continuous flow of a suspension of cells to be electroporated;
5 an inlet flow portal in fluid communication with the flow channel, whereby the suspension can be introduced into the flow channel through the inlet flow portal;
an outlet flow portal in fluid communication with the flow channel, whereby the suspension can be withdrawn from the flow channel through
10 the outlet portal;
the walls defining the flow channel within the electroporation zone comprising a first electrode forming a substantial portion of a first wall of the flow channel and a second electrode forming a substantial portion of a second wall of the flow channel opposite the first wall, the first and second
15 electrodes being such that when placed in electrical communication with a source of electrical energy an electric field is formed therebetween through which the suspension can flow; and
wherein the thermal resistance of the flow channel is less than approximately 10°C per Watt.
20
44. The device of Claim 43, wherein the thermal resistance of the flow channel is less than approximately 4°C per Watt.
45. The device of Claim 43, wherein the thermal resistance of
25 the flow chamber is approximately 0.1°C per Watt to 4°C per Watt.
46. The device of Claim 43, wherein the thermal resistance of the flow chamber is approximately 1.5°C per Watt to 2.5°C per Watt.
47. The device of Claim 43, wherein the first and second
30 electrodes are spaced from each other at least 1 mm.

48. The device of Claim 43, wherein the flow chamber has a ratio of combined electrode surface in contact with buffer to the distance between the electrodes of approximately 1 to 100.

5 49. The device of Claim 43, wherein the cells electroporated in the flow channel are not substantially thermally degraded thereby.

50. A flow electroporation device comprising:
a flow chamber for containing a suspension of cells to be
10 electroporated;
the flow chamber being at least partially defined by opposing oppositely chargeable electrodes; and
wherein the flow chamber has a ratio of combined electrode surface in contact with buffer to the distance between the electrodes of
15 approximately 1 to 100.

51. The device of Claim 50, wherein the ratio is approximately 1 to 90.

20 52. The device of Claim 50, wherein the ratio is approximately 1 to 80.

53. The device of Claim 50, wherein the ratio is approximately 1 to 70.
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54. The device of Claim 50, wherein the ratio is approximately 1 to 60.

55. The device of Claim 50, wherein the ratio is approximately 1 to 50.
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56. A flow electroporation device comprising:
walls defining a flow channel configured to receive and to transiently contain a continuous flow of a suspension of cells to be electroporated;
5 an inlet flow portal in fluid communication with the flow channel, whereby the suspension can be introduced into the flow channel through the inlet flow portal;
an outlet flow portal in fluid communication with the flow channel, whereby the suspension can be withdrawn from the flow channel through
10 the outlet portal;
the walls defining the flow channel comprising a first electrode forming at least a portion of a first wall of the flow channel and a second electrode forming at least a portion of a second wall of the flow channel opposite the first wall, the first and second electrodes being such that when
15 placed in electrical communication with a source of electrical energy an electric field is formed therebetween through which the suspension can flow; and
wherein the thermal resistance of the flow channel is less than approximately 10°C per Watt.
20
57. The device of Claim 56, wherein the thermal resistance of the flow channel is less than approximately 4°C per Watt.
58. The device of Claim 56, wherein the thermal resistance of
25 the flow channel is approximately 0.1°C per Watt to 10°C per Watt.
59. The device of Claim 56, wherein the thermal resistance of the flow channel is approximately 1.5°C per Watt to 2.5°C per Watt.
- 30 60. The device of Claim 56, wherein the first and second electrodes are spaced from each other at least 1 mm.

61. The device of Claim 56, wherein the first and second electrodes are spaced from each other at least 3 mm.

5 62. The device of Claim 56, wherein the flow chamber has a ratio of combined electrode surface in contact with buffer to the distance between the electrodes of approximately 1 to 100.

10 63. The device of Claim 56, wherein the flow chamber has a ratio of combined electrode surface in contact with buffer to the distance between the electrodes of approximately 1 to 100 and wherein the first and second electrodes are spaced from each other at least 1 mm.

15 64. The device of Claim 56, wherein the flow chamber has a ratio of combined electrode surface in contact with buffer to the distance between the electrodes of approximately 1 to 100 and wherein the first and second electrodes are spaced from each other at least 3 mm.

20 65. The device of Claim 56, wherein the flow chamber has a ratio of combined electrode surface in contact with buffer to the distance between the electrodes of approximately 1 to 100 and wherein the first and second electrodes are spaced from each other approximately 3 mm to approximately 2 cm.

25 66. The device of Claim 56, wherein the cells electroporated in the flow channel are not substantially thermally degraded thereby.

67. A flow electroporation device, comprising:

walls defining a flow channel configured to receive and to transiently contain a continuous flow of a suspension comprising particles;

5 an inlet flow portal in fluid communication with the flow channel, whereby the suspension can be introduced into the flow channel through the inlet flow portal;

an outlet flow portal in fluid communication with the flow channel, whereby the suspension can be withdrawn from the flow channel through the outlet flow portal;

10 the walls defining the flow channel comprising a first electrode plate forming a first wall of the flow channel and a second electrode plate forming a second wall of the flow channel opposite the first wall; wherein the area of the electrodes contact with the suspension, and the distance between the electrodes is chosen so that the thermal resistance of the flow channel is less than approximately 4°C per Watt.

15 the paired electrodes placed in electrical communication with a source of electrical energy, whereby an electrical field is formed between the electrodes;

20 whereby the suspension of the particles flowing through the flow channel can be subjected to an electrical field formed between the electrodes.

25

5 68. The flow electroporation device of Claim 67, wherein the electrode plates defining the flow channel further comprises: a gasket formed from an electrically non-conductive material and disposed between the first and second electrode plates to maintain the electrode plates in spaced-apart relation, the gasket defining a channel therein forming opposed side walls of the flow channel.

10 69. The flow electroporation device of Claim 67, wherein the gasket forms a seal with each of the first and second electrode plates.

15 70. The flow electroporation device of Claim 67, wherein the device comprises a plurality of flow channels, and wherein the gasket comprises a plurality of channels forming opposed side walls of each of the plurality of channels.

20 71. The flow electroporation device of Claim 67, wherein one of the inlet flow portal and the outlet flow portal comprises a bore formed in one of the electrode plates and in fluid communication with the flow channel.

25 72. The flow electroporation device of Claim 67, wherein the other of the inlet flow portal and the outlet flow portal comprises a bore formed in the one of the electrode plates and in fluid communication with the flow channel.

30 73. The flow electroporation device of Claim 67, wherein the other of the inlet flow portal and the outlet flow portal comprises a bore formed in the other of the electrode plates and in fluid communication with the flow channel.

74. The flow electroporation device of Claim 67, further comprising a cooling element operatively associated with the flow channel to dissipate heat.

5 75. The flow electroporation device of Claim 67, wherein the cooling element comprises a thermoelectric cooling element.

76. The flow electroporation device of Claim 67, wherein the cooling element comprises a cooling fluid flowing in contact with the electrode.
10

77. The flow electroporation device of Claim 67, wherein the cooling element comprises a heat sink operatively associated with the electrode.
15

78. The flow electroporation device of Claim 67, wherein the heat resistance of the flow channel is less than approximately 3°C per watt.

20 79. The flow electroporation device of Claim 67, wherein the heat resistance of the flow channel is less than approximately 2°C per watt.

80. The flow electroporation device of Claim 67, wherein the heat resistance of the flow channel is between approximately 0.5 °C per Watt and 4°C per Watt.
25

81. The flow electroporation device of Claim 67, wherein the heat resistance of the flow channel is between approximately 1°C per Watt and 3°C per Watt.
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82. The flow electroporation device of Claim 67, wherein the heat resistance of the flow channel is between approximately 1.5°C and 2.5°C.

5 83. The flow electroporation device of Claim 67, wherein the first electrode comprises an elongated, electrically conductive structure,

wherein the second electrode comprises a tubular, electrically conductive structure;

10 wherein the electrodes are concentrically arranged such that the second, tubular electrode surrounds the first electrode in spaced-apart relation thereto; and

wherein the flow channel is disposed within an annular space defined between the first and second electrodes.

15 84. The flow electroporation device of Claim 83, wherein the electrodes form at least a portion of the walls defining the flow channel.

20 85. The flow electroporation device of Claim 83, further comprising concentric annular spacers for maintaining the first and second electrodes in spaced-apart, concentric relation.

25 86. The flow electroporation device of Claim 83, wherein the device is arranged in series with a second, like device.

87. The flow electroporation device of Claim 83, wherein the device is arranged in parallel with a second, like device.

5 88. A method of transfecting a cell comprising providing a nucleic acid or an expression vector coding for a desired protein or peptide and introducing the expression vector into the cell by flow electroporation using a flow channel having a thermal resistance of less than approximately 10°C per Watt.

10 89. The method of Claim 88, wherein the thermal resistance of the flow channel is less than approximately 4°C per Watt.

90. The method of Claim 88, wherein the thermal resistance of the flow channel is approximately 0.1°C per Watt to 4°C per Watt.

15 91. The method of Claim 88, wherein the thermal resistance of the flow channel is approximately 1.5°C per Watt to 2.5°C per Watt.

20 92. The method of Claim 88 wherein greater than 50% of the cells transfected by electroporation express the desired protein or peptide.

25 93. The method of Claim 88 wherein approximately 50% to 95% of the cells transfected by electroporation express the desired protein or peptide.

30 94. The method of Claim 88, wherein approximately 60% to 90% of the cells transfected by electroporation express the desired protein or peptide.

95. The method of Claim 88, wherein approximately 70% to 80% of the cells transfected by flow electroporation express the desired protein or peptide.

5 96. The method of Claim 88, wherein the cells transfected by flow electroporation are greater than approximately 50% viable.

10 97. The method of Claim 88, wherein the cells transfected by flow electroporation are greater than approximately 60% viable.

15 98. The method of Claim 88, wherein the cells transfected by flow electroporation are greater than approximately 70% viable.

20 99. The method of Claim 88, wherein the cells transfected by flow electroporation are greater than approximately 80% viable.

 100. The method of Claim 88, wherein the cells transfected by flow electroporation are greater than approximately 90% viable.

25 101. The method of Claim 88, wherein the cells transfected by flow electroporation are approximately 50% to 90% viable.

30 102. The method of Claim 88, wherein the cells transfected by flow electroporation are approximately 60% to 90% viable.

103. The method of Claim 88, wherein the cells transfected by flow electroporation art approximately 70% to 80% viable.

5 104. The method of Claim 88, where and the desired nucleic acid or protein is b-cell differentiation factor, b-cell growth factor, mitogenic cytokine, chemotactic cytokine, colony stimulating factor, angiogenesis factor, cadherin, selectin, integrin, NCAM, ICAM, L1, t-cell replacing factors, differentiation factor, transcription factor, mRNA, heat shock
10 protein, nuclear protein complexe, RNA/DNA oligomer, IFN-alpha, IFN-beta, IFN-omega, IL1, IL2, IL3, IL4, IL5, IL6, IL7, IL8, IL9, IL10, IL11, IL12, IL13, IL14, IL15, IL16, IL17, IL18, leptin, myostatin, macrophage stimulating protein, platelet-derived growth factor, TNF-alpha, TNF-beta, NGF, CD40L, CD137L/4-1BBL, human lymphotoxin-beta, TNF-related
15 apoptosis-inducing ligand, monoclonal antibody, fragments of monoclonal antibody, G-CSF, M-CSF, GM-CSF, PDGF, IL1-alpha, IL1-beta, FGF IFN-gamma, IP-10, PF4, GRO, 9E3, erythropoietin, endostatin, angiostatin, fibroblast growth factor, VEGF, or soluble receptor and any fragments or combinations thereof.

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105. The method of Claim 88, wherein the desired nucleic acid or protein is erythropoietin or fragments thereof.

25 106. The method of Claim 88, wherein the desired nucleic acid or protein is endostatin or fragments thereof.

107. The method of Claim 88, wherein the desired nucleic acid or protein is angiostatin or fragments thereof.

30 108. The method of Claim 88, wherein the desired nucleic acid or protein is IL12 or fragments thereof.

109. The method of Claim 88, wherein the desired nucleic acid or protein is IL2 or fragments thereof.

5 110. The method of Claim 88, comprising the further step of administering the transfected cells to a patient.

111. A method of delivering a therapeutic agent to patient comprising:

10 incorporating the therapeutic agent into platelets by electroporation using a flow electroporation channel having a thermal resistance of less than approximately 10°C per Watt; and administering the platelets to the patient.

15 112. The method of Claim 111, wherein the thermal resistance of the flow channel is less than approximately 4°C per Watt.

20 113. The method of Claim 111, wherein the thermal resistance of the flow channel is approximately 0.1°C per Watt to 4°C per Watt.

25 114. The method of Claim 111, wherein the thermal resistance of the flow channel is approximately 1.5°C per Watt to 2.5°C per Watt.

115. The method of Claim 111, wherein the platelets are administered to the patient intravenously.

116. The method of claim 111, wherein the therapeutic agent is AGM-1470 (TNP-470), MetAP-2; growth factor antagonists, antibodies to growth factors; growth factor receptor antagonists; TIMP, batimastat, marimastat; genistein SU5416; alphaVbeta3/5, retinoic acid fenretinide, 5 11 -epihydrocortisol, corteloxone, tetrahydrocortisone and 17 -hydroxyprogesterone; staurosporine, MDL 27032; 22-oxa-1 alpha, and 25-dihydroxyvitamin D3; indomethacin and sulindac; minocycline; thalidomide and thalidomide analogs and derivatives; 2-methoxyestradiol; tumor necrosis factor-alpha; interferon-gamma-inducible protein 10 (IP-10); interleukin 1 and interleukin 12; interferon alpha, beta or gamma; 10 angiostatin protein or plasminogen fragments; endostatin protein or collagen 18 fragments; proliferin-related protein; group B streptococcus toxin; CM101; CAI; troponin I; squalamine; L-NAME; thrombospondin; wortmannin; amiloride; spironolactone; ursodeoxycholic acid; bufalin; 15 suramin; tecogalan sodium; linoleic acid; captopril; irsogladine; FR-118487; triterpene acids; castanospermine; leukemia inhibitory factor; lavendustin A; platelet factor-4; herbimycin A; diaminoantraquinone; taxol; aurintricarboxylic acid; DS-4152; pentosan polysulphite; radicicol; fragments of human prolactin; erbstatin; eponemycin; shark cartilage; 20 protamine; Louisianin A, C and D; PAF antagonist WEB 2086; auranofin; ascorbic ethers; or sulfated polysaccharide D 4152.

117. A method of treating a patient with a therapeutic nucleic acid, protein or peptide comprising:

transfecting a cell population with a nucleic acid or an expression vector that codes for the desired protein or peptide by flow electroporation;

administering a therapeutically effective amount of the transfected cells to the patient.

118. The method of Claim 117, wherein the therapeutic nucleic acid, protein or peptide is b-cell differentiation factor, b-cell growth factor, mitogenic cytokine, chemotactic cytokine, colony stimulating factor, angiogenesis factor, cadherin, selectin, integrin, NCAM, ICAM, L1, t-cell replacing factors, differentiation factor, transcription factor, mRNA, heat shock protein, nuclear protein complex, RNA/DNA oligomer, IFN-alpha, IFN-beta, IFN-omega, IL1, IL2, IL3, IL4, IL5, IL6, IL7, IL8, IL9, IL10, IL11, IL12, IL13, IL14, IL15, IL16, IL17, IL18, leptin, myostatin, macrophage stimulating protein, platelet-derived growth factor, TNF-alpha, TNF-beta, NGF, CD40L, CD137L/4-1BBL, human lymphotoxin-beta, TNF-related apoptosis-inducing ligand, monoclonal antibody, fragments of monoclonal antibody, G-CSF, M-CSF, GM-CSF, PDGF, IL1-alpha, IL1-beta, FGF IFN-gamma, IP-10, PF4, GRO, 9E3, erythropoietin, endostatin, angiostatin, fibroblast growth factor VEGF, or soluble receptor and any fragments or combinations thereof.

119. The method of Claim 117, wherein the desired protein is erythropoietin or fragments thereof.

120. The method of Claim 117, wherein the desired protein is endostatin or fragments thereof.

121. The method of Claim 118, wherein the desired protein is angiostatin or fragments thereof.

122. The method of Claim 119, wherein the desired protein is IL12 or fragments thereof.

5 123. The method of Claim 119, wherein the desired protein is IL2 or fragments thereof.

124. A method comprising:
flowing a suspension of particles to be electroporated
10 through a flow channel; and
exposing said suspension of particles to an electric field while flowing through said flow channel, said electric field having a strength of greater than 0.5 kV/cm.

15 125. The method of Claim 124, wherein said electric field has a strength of greater than approximately 3.5 kV/cm.

126. The method of Claim 124, wherein said particles are CLL-B cells.

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127. A flow electroporation device comprising:

walls defining a flow channel configured to receive and to transiently contain a continuous flow of a suspension of cells to be electroporated;

5 an inlet flow portal in fluid communication with the flow channel, whereby the suspension can be introduced into the flow channel through the inlet flow portal;

an outlet flow portal in fluid communication with the flow channel, whereby the suspension can be withdrawn from the flow channel through the outlet portal;

10 the walls defining the flow channel comprising a first electrode forming at least a portion of a first wall of the flow channel and a second electrode forming at least a portion of a second wall of the flow channel opposite the first wall, the first and second electrodes being such that when placed in electrical communication with a source of electrical energy an electric field is formed therebetween through which the suspension can flow; and

15 wherein said first electrode is spaced from said second electrode by a distance greater than 3 mm.

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128. A flow electroporation device of Claim 127, wherein said first electrode is spaced from said second electrode by a distance of approximately 4 mm to 2 cm.

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129. A flow electroporation device of Claim 127, wherein said first electrode is spaced from said second electrode by a distance of approximately 5 mm to 1 cm.

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